IDEXX COLILERT®-18 TEST METHOD FOR THE SIMULTANEOUS DETECTION OF TOTAL COLIFORMS AND *E. COLI* IN WATER

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1. Scope and Application

1.1. This method is intended for use in the simultaneous detection and confirmation of total coliforms and *E. coli* in water. Any positive sample for total coliforms is an indication of contamination. Any positive sample for both total coliforms and *E. coli* is an acute violation.

1.2. The minimum, non-zero number of bacterial counts detectable with this method is a function of the dilution scheme used when processing the sample.

1.3. The Colilert-18 method can be applied to fresh waters, drinking waters, ground waters, reuse waters, waste waters and marine waters. It can be used as a Presence/Absence test or quantification with the Quanti-Tray® system (20.1, 20.5) or with 5 X 20 mL, 10 X 10mL, 15 tubes serial dilution (MPN).

1.4. Since there can be wide range of coliform levels in surface waters and wastewaters, dilutions can be used with this method for detecting and enumerating the actual level.

1.5. Colilert-18 can be used for detecting *E. coli* in marine waters (not for total coliforms, unless evaluated by the specific site). Make at least a 1:10 dilution using sterile non-buffered oxidant free water for *E. coli*. In some sub-tropical waters, a 1:20 dilution may be necessary.

2. Summary of Method

2.1. The method is based on Defined Substrate Technology®. The product utilizes nutrient indicators that produce color/fluorescence when metabolized by total coliforms and *E. coli*. When the reagent is added to the sample and incubated, it can detect these bacteria at 1 CFU/ 100 mL at 18 hours and up to 22 hours with as many as 2 million heterotrophic bacteria/100 mL present.

2.2. Colilert-18 is in *Standard Methods for the Examination Water and Wastewater*, On-line and in the 20th, 21st & 22nd edition AWWA, APHA, WEF, 9223B (20.3)

3. Definitions

3.1. In this method, coliform bacteria are those bacteria which produce a yellow color and for *E. coli*, also produce a fluorescent signal under a 6 watt, 365-366 nm UV light after incubation at 35 ± 0.5°C at 18 hours and up to 22 hours with as many as 2 million heterotrophic bacteria/100 mL present.

4. Interferences

4.1. Some samples containing humic material may have an innate color and a control blank of the same water sample may be required for comparison to the inoculated sample or a dilution may be made.

4.2. Heterotrophic bacteria greater than 2,000,000/100mL could yield a positive coliform reaction for coliforms.

5. Safety

5.1. The analyst/technician must know and observe the normal safety procedures required in a microbiology laboratory preparing, using, and disposing of samples, reagents and materials, and while operating sterilizing equipment.

5.2. Mouth pipetting is prohibited.

6. Equipment and Supplies

6.1. Sterile Pipettes, sterile, T.D. bacteriological or Mohr, glass or plastic of appropriate volume and sterile loops.

6.2. Sterile vessels, glass or plastic (free from fluorescence) with or without sodium thiosulfate. Any water containing an oxidizing agent such as chlorine must be neutralized with sodium thiosulfate.

6.2.1. Vessels containing sodium thiosulfate must neutralize up to 5 mg/L of chlorine for drinking water samples and up to 15 mg/L for waste water effluents.

6.2.2. Vessels must be at least 120 mL or of larger capacity to hold 100 mL sample to allow for proper mixing of sample.

6.3. 51 well Quanti-Tray or Quanti-Tray/2000

6.4. Quanti-Tray Sealer

6.5. Incubator maintained at 35 ± 0.5°C.

6.6. 6 Watt 365-366 nm UV light.

7. Reagents

7.1. Sterile, non-buffered, oxidant-free water for dilutions. (20.1)

7.2. Presence /Absence**,** 51 Well Quanti-Tray or Quanti-Tray 2000 Comparator.

7.3. Antifoam reagent (optional).

7.4. Sodium thiosulfate reagent *Standard Methods for the Examination of Water and Wastewater*, (20.3) or sterile vessels containing sodium thiosulfate to neutralize up to 15mg/L chlorine.

7.5. Store Colilert-18 at 2-25°C away from light. The expiration date is indicated on the package (15 months from the date of manufacture).

8. Sample Collection, Preservation and Storage

8.1. Sampling procedures as described in detail in the USEPA microbiology methods manual, Section II, A (20.2) and in Standard Methods for the Examination of Water and Wastewater (20.3).

8.1.1. Storage Temperature and Handling Conditions: Ice or refrigerate bacteriological samples at a temperature less than 10°C (2-10°C) during transit to the laboratory. Use insulated containers to assure proper maintenance of storage temperature. Ensure that sample vessels are not totally immersed in water during transit. Do not allow samples to freeze. If frozen, sample cannot be thawed and a new sample is required.

8.1.2. Holding Time Limitations: Examine samples as soon as possible after collection. For drinking water samples do not exceed 30 hours hold time from collection to incubation. For non-potable water for compliance, do not exceed 8 hours from time of collection to incubation (20.4).

9. Quality Control

9.1. Quality control should be conducted on each lot of Colilert-18 or more often as regulations requires. One of the following quality control procedures is recommended for each lot of Colilert-18 when used for total coliform and *Escherichia coli* testing:

9.1.1. IDEXX-QC Coliform and *E. coli*: Consists of 3 each of *Escherichia coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*

1. See the package insert for instructions.
2. Obtain the mean and range from the website; www.idexx.com/water under Quality Certificates.

9.1.2. Quanti-Cult: Consists of 3 each of *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.*

1. See package insert for instructions.

9.1.3. **ATCC:** Fill three sterile vessels with 100 *mL* of sterile non-buffered oxidant-free water and inoculate with a sterile loop of ATCC strains, *Escherichia coli* ATCC 25922 or 11775, *Klebsiella pneumoniae* ATCC 31488 and *Pseudomonas aeruginosa* ATCC 10145 or 27853.

9.2. Follow Section 12.0 P/A Procedure or Section 13.0 Quanti-Tray Enumeration Procedure and Section 14.0 Interpretation and Calculations.

9.3. Sample bottle and Quanti-Tray sterility check per lot (20.7; see Section V, 5.4.2)

9.3.1. At least one sample bottle/lot and tray/lot are tested with Tryptic Soy Both (25 mL for bottles and 100 mL for trays) and incubated at 35±0.5°C. It is recommended that this be performed in a laminar flow hood. Aseptic technique must be used. If not available, aseptic technique must be maintained. Do not open bottle for long periods of time nor place the cap on the lab surface facing up. Open cap just enough to add the TSA to the bottle and close immediately.

9.3.2. Check samples at 24 and 48 hours for growth.

9.3.3. No growth should be observed.

9.3.4. If growth is observed, retest and if still positive, call IDEXX Water Technical Service (1-800-321-0207).

9.4. Monthly Sealer Check with food color or dye: (20.7; see Section V, 5.3.2.1.2)

9.4.1. Add 2-3 drops of food coloring dye or equivalent to 100 mL of water. Mix well.

9.4.2. Add this to the Quanti-Tray and seal the tray.

9.4.3. Observe the tray. There should be no dye observed outside the wells.

9.4.4. If dye is observed outside the well, repeat the testing and if it still occurs call IDEXX Water Technical Service (1-800-321-0207).

9.5. Media sterility check using sterile water per lot and vessels do not auto-fluoresce (20.7; Section V, 5.3.1.3)

9.5.1. Each new lot shall be checked for sterility. Select at least one vessel and one blister pack and add 100 mL of sterile DI water. Mix well and incubate up to18 and no longer than 22 hours at 35±0.5°C

9.5.2. No color or fluorescence should be observed.

9.5.3. If color and/or fluorescence is observed, retest and if still positive, call IDEXX Water Technical Service (1-800-321-0207).

10. Calibration and Standardization

10.1. Check the temperature of the incubator at least twice per day (when in use) separated by at least 4 hours to insure it is within the stated limits. Record the date, temperature, time of reading and initial.

10.2. Check thermometers at least annually against NIST certified thermometer or one that meets the requirements of NIST Monograph SP 250-23 (20.3).

11. Corrective Action

11.1. If an unacceptable result is obtained, then the lab should review the test procedure to determine the cause of the failure to prevent this from reoccurring again by:

11.2. Defining the problem:

A. Identify corrective action and steps required to correct the problem.
B. Implement correction action.
C. Document corrective action.

11.3. Repeat testing to ensure that corrective action was successful.

11.4. Examples are:

11.4.1. Procedure followed for preparing the control and or diluent.
11.4.2. Incubation temperature within the required tolerance.
11.4.3. Verified the thermometer for the incubator or water bath was calibrated against NIST thermometer and corrections made if required. (20.3)
11.4.4. Sample incubation within the required time period.
11.4.5. Test kit is within the expiration date.
11.4.6. Call and review problem encountered with IDEXX Water Technical Service at 1-800-321-0207.

12. Presence-Absence (P/A) Procedure

12.1. Carefully separate one blister pack from the strip taking care not to accidentally open the adjacent pack.

12.2. Ensure the powder is in the bottom of the blister pack.

12.3. Hold the blister pack face down (paper side up) at the top and towards the bottom and snap back at the score line forming a “V”, with the opening facing into the open vessel.

12.4. Allow the powder to fall into the water sample contained in the sterile, non-fluorescent vessel.

12.5. Aseptically cap and seal the vessel.

12.6. Mix well until dissolved.

12.7. If sample is not already at 33-38°C, then place vessel in a 35°C water bath for 20 minutes or alternatively, at 44.5°C water bath for 7-10 minutes (this is part of the 18 hour incubation).

12.8. Incubate for 18 and up to 22 hours at 35 ± 0.5°C.

12.9. Read the results at 18 hours. In addition, laboratories may incubate samples for additional time (up to 22 hours total for their convenience). Compare each result against the comparator dispensed into an identical vessel.

12.10. If less yellow than the comparator, the test is negative. **Note:** However, *if the results are ambiguous to the analyst based on the initial reading, incubate up to an additional four hours (but not to exceed 22 hours total) to allow the color to intensify*.

12.11. If the sample has a yellow color equal to or greater than the comparator, the presence of total coliforms is confirmed. If color is not uniform, mix by inversion, then recheck.

12.12. If yellow is observed, check vessel for fluorescence by placing a 6-watt, 365-366 nm UV light within five inches of the sample in a dark environment. Be sure the light is facing away from your eyes and towards the vessel. If the fluorescence is equal to or greater than the comparator, the presence of *E. coli* is confirmed.

12.13. Positives for both total coliforms and *E. coli* observed before 18 hours and negatives observed after 22 hours are also valid.

13. Quantification Procedure

13.1. For accuracy and counting range, use the IDEXX Quanti-Tray System with either the 51 Well Quanti-Tray or the Quanti-Tray/2000 and follow the above Presence-Absence procedure (12.1-12.6). The use of IDEXX Antifoam may be necessary to reduce foaming and eliminate excess bubbles in the wells of the Quanti-Tray. If not, allow the foam to dissipate for 30-60 seconds prior to adding the sample to the tray. Note; all foam does not need to be dissipated. It can be used for multiple tube (MPN); 5 tubes X 20 mL, 10 tubes X 10 mL, or 15 tube serial dilutions. Consult Standard Methods for the Examination of Water and Wastewater for the appropriate MPN Tables.

13.2. If a dilution is required, use sterile deionized or distilled water, not buffered water for making the dilutions. Always add Colilert-18 to the final 100 mL diluted sample only.

13.3. Follow the package insert for the Quanti-Tray (20.5) along with the package insert for Colilert-18 (20.1) and/or see 12.1-12.6 above. Remove a sterile tray from the plastic bag (tear open the plastic bag at the bottom which has a black line around the bag) and remove the number of trays required for testing. Close the bag using tape or a clip. Label the back of the tray with a felt tip marker to identify the sample. Open the tray following the directions as outlined in the insert for Quanti-Tray (20.5). Pour the room temperature sample reagent mixture from the vessel into the tray avoiding contact with the foil tab. Seal the tray with the Quanti-Tray sealer.

13.4. Incubate at 35± 0.5°C for 18 and up to 22 hours. Pre-warming is not required. See package insert for instructions.

14. Interpretation and Calculations

14.1. Follow the same interpretation directions from Section 12.9 -12.12 to count the number of positive wells. Refer to the Quanti-Tray MPN Table provided

by IDEXX to determine the Most Probable Number (MPN) for total coliforms (yellow wells) and *E. coli* (yellow and fluorescent wells) in the sample. Correct the MPN value for any dilution made. The color and fluorescence of positive wells may vary. Use the appropriate Quanti-Tray MPN comparator following the instructions as indicated.

14.2. Positives for both total coliforms and *E. coli* observed before 18 hours and negatives observed after 22 hours are also valid.

15. Method Performance

15.1. Colilert-18 found equally as sensitive compared to LTB, BGLB and EC + MUG. E. coli recovery was not statistically different compared to m-TEC. (20.6).

16. Reporting Results

16.1. Quantification, report results as MPN/100 mL for total coliforms and *E. coli*

17. Verification Procedure

17.1. Not applicable

18. Pollution Prevention

18.1. The solutions and reagents used in this method pose no threat to the environment when recycled and managed properly.

18.2. Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed.

19. Waste Management

19.1. It is the laboratory's responsibility to comply with all federal, state and local regulations governing waste management, particularly the biohazard and hazardous identification rules and land disposal regulations. Compliance with all sewage discharge permits and regulations is also required.

19.2. Samples, reference materials and equipment known or suspected to have viable bacteria attached or contained must be sterilized prior to disposal.

20. References

20.1. Colilert-18 Package Insert from IDEXX.

20.2. Bordner, R., J.A. Winter and P.V. Scarpino (eds.) Microbiological Methods for Monitoring the Environment, Water and Wastes, EPA-600/8-78-017. Office of Research and Development, USEPA. (December 1978)

20.3. Clesceri, L.S., A.E. Greenberg, A.D. Eaton (eds.). 1998 Standard Methods for the Examination of Water and Wastewater, 20th Edition, American Public Health Association, Washington, DC (also see 21st, 22nd and on-line edition)

20.4. Federal Register/ Vol 77, #97/ Friday, May 18th 2012, page 29806-29807

20.5. Quanti-Tray Package Insert from IDEXX

20.6. Federal Register/vol.66, No. 169 / Thursday, August 30th, 2001, page 45818

20.7. USEPA Manual for Certification of Laboratories Analyzing Drinking Water, Fifth Edition, Section V.